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The Impact of Modern Processed and Natural Food Diets on Flavor-Nutrient Learning and Response to Sweet Taste in Rats

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by

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Abstract

Animals use classical conditioning to learn predictive relationships between flavors and postingestive nutrients, which allows them to regulate their body weights. This is made difficult by modern diets, which have confusing flavor-nutrient relationships due to added fats, sugars, and flavors in processed foods. Cafeteria diets containing a variety of human-typical foods are often used to study the effects of a modern diet in animal models. Foods used in cafeteria diets typically combine aspects such as high-fat high-sugar, variety, and high palatability. However, no past studies have analyzed the effect of variety on flavor-nutrient learning by using only natural foods. In the current study, 36 rats were assigned to three dietary conditions: a processed foods (PF) cafeteria diet, natural foods (NF) cafeteria diet, or chow-only control (CON) diet. After three months on the diets, rats were tested on their ability to learn about new foods and on their response to sweet taste. The rats were first tested with flavor-nutrient conditioning (FNC) to analyze the degree to which they were capable of learning new flavor-nutrient relationships. Several measures of FNC revealed that PF rats were not impaired in learning, and were perhaps better able to discriminate between flavors than NF or CON rats. Throughout the present studies, rats in the cafeteria diet groups were found to consistently consume less sweet-tasting solutions than CON rats in *ad libitum* intake tests. To determine the cause of this difference in sweet intake, rats' motivation and hedonic liking for sucrose was analyzed by using a progressive ratio lever-pressing task for sucrose reward as well as lick microstructure analysis. Results indicated that rats were all equally

motivated to work for sucrose, but that NF rats perceived high concentrations of sucrose as much more palatable than PR and CON rats. This study demonstrates that processed and natural foods cafeteria diets do not impair new flavor-nutrient learning, but they do cause rats to reduce sugar intake, for which the reason is still unknown.

The Impact of Modern Processed and Natural Food Diets on Flavor-Nutrient Learning and Response to Sweet Taste in Rats

This thesis will be concerned with the impact of processed and natural food cafeteria diets on flavor-nutrient learning and the reward value of sugar in rats. In the introduction, I will first review how different types of flavor-nutrient inconsistency, typical of modern processed diets, cause behavioral changes and weight gain. I will then discuss the effects of a useful tool for studying behavioral modifications caused by a modern diet: the cafeteria diet. Finally, I will explain the methods of our experiment, which researched the effects of variety and flavor-nutrient confusion within a cafeteria diet in addition to determining the cafeteria diet's impact on perceived sugar reward.

In modern consumer-based societies, overeating and consequential obesity are important problems. One relevant cause of this health crisis is the modern human diet and its discrepancy with evolved human and animal behavior. In our ancestors' past, capitalizing on all available foods was an advantageous strategy. Most foods were of low quality and difficult to obtain, and the next meal was probably uncertain. Foods containing higher levels of calories, especially those with fats or sugars, were prized for their energy. Animals, including human ancestors, evolved to prefer nutrient-dense foods that were important for survival. Foods rich in fats and sugars became extremely palatable to humans, and today fats and sugars are still extremely well-liked by humans. Studies show that combining sweet taste with high levels of fats produces extremely high hedonic

responses in humans that are greater than the hedonic value achieved by either fats or sugars alone (Drewnowski & Greenwood, 1983). Taste sensitivity is a genetically-controlled trait that causes each human to have a unique sensitivity and reaction to certain tastes such as bitterness (Krebs, 2009). However, there are some intrinsic preferences that are present at birth in all humans and animals. Newborn humans and rats differentiate between sweet and non-sweet flavors, and show an innate positive reaction to sweet tastes (Rosenstein & Oster, 1988; Hall & Bryan, 1981).

In countries such as the United States where food is plentiful for most people, humans no longer have the need to exploit all nutrient-dense food sources that are encountered. However, we have retained from our ancestral past the inherent liking for fats and sugars. Our evolved preference for foods rich in rats and sugars is an evolutionary mismatch with the overabundance and easy access to unhealthy "junk foods" of modern society. One problem with the vestigial behavior of preferring and seeking out high-fat and high-sugar foods is that it causes overeating, which can lead to eventual obesity (Birch 1999). A more complicated problem that has arisen, however, is the modern relationship with processed foods.

Many of the modern foods that are rich in fats and sugars belong to the category of processed foods. "Processed" means that the foods are highly modified from their natural ingredients, with added ingredients, fats, sugars, and flavors. The levels of fats and sugars that exist in processed foods are much higher than levels that any food would contain in the wild. For example, common

fruits such as apples, pears, blueberries, and grapes are between 8 and 15% sugar by weight. The sugar content of processed foods is much higher; apple granola is 26% sugar, cherry pie filling is 22% sugar, and pumpkin muffins can be as high as 32% sugar by weight.

Modified eating behaviors associated with processed foods, whose added fats and carbohydrates are rapidly absorbed by the body, have been found to mimic behaviors related to addiction (Schulte, Avena, & Gearhardt, 2015). Much like drugs, foods that are processed are much more capable of causing addictions and being abused (Schulte, Avena, & Gearhardt, 2015). Processed foods that are high in fats and sugars become supernormal stimuli in terms of feeding behavior; these foods, which are so much more palatable than the foods that animals have evolved to seek out and consume, induce extreme reactions that are exaggerations of reactions to natural levels of fats and sugars.

Artificial flavors and other processing techniques that alter the flavor, texture, and other sensory characteristics of food are a possible cause of important health-related changes because they have the potential to impede straightforward flavor-nutrient relationships that humans learn as they grow up and gain experience with a variety of foods. In nature, raspberry flavor in an animal's mouth signifies that the animal is eating a raspberry and will soon experience the sugary postingestive effects that raspberries consistently produce. However, modern grocery stores boast countless raspberry-flavored products that are completely unrelated to the natural product. Raspberry-flavored granola, cookies, gum, drinks, and more all taste like raspberries, but these different products are

each associated with vastly different nutritional consequences in the gut. Flavor-nutrient inconsistencies such as these are a recent but huge phenomenon. A history of flavor confusion could be partly responsible for disrupting humans' natural learning about foods, contributing to the current obesity problem.

One indication that learning flavor-nutrient relationships has been disrupted might be increased weight gain. One research group put rats on four different diets of flavored rat chow that was diluted with cellulose to three possible caloric densities. Each group had a different level of consistency of whether flavors reliably indicated the caloric density of the food being consumed (Warwick & Schiffman, 1991). The control group received one consistent mid-density food that was always paired with one consistent flavor. The "density variety" group received one of three caloric density chows each day, and each of the three densities had its own consistent flavor. The third group, on the "flavor variety" diet, always received the same mid-density chow, but the chow was flavored differently on different days. Finally, the most inconsistent flavor-nutrient group, called the "novel" group, randomly received one of three chow densities each day, and the chow was randomly paired with a different flavor every day. The food and flavors in this group were unpaired, and thus rats could not predict from the flavor which density of chow they were eating. Rats in this most inconsistent flavor-nutrient group gained the greatest amount of body weight (Warwick & Schiffman, 1991). These significant results, which indicate that unpredictable flavor/calorie relationships inhibit an animal's ability to regulate its body weight, were produced by a diet that manipulated only caloric density as

related to flavor. Additional sensory inconsistencies could cause an even greater effect.

Other studies support the hypothesis that decreasing the reliability of food cues makes body weight regulation difficult. Davidson and Swithers (2004) gave rats experience with an inconsistent relationship between sweet taste and calories by providing them with alternating sweet caloric solutions and sweet, artificially non-caloric solutions. Rats trained with this inconsistent sweet-calorie relationship were not able to compensate for liquid calories by adjusting their food intake. In comparison, rats that were always exposed to sweet solutions that consistently predicted a natural level of calories were better able to adjust total caloric intake to maintain body weight homeostasis (Davidson & Swithers, 2004). This same concept of inconsistent flavor/calorie relationships was explored by Swithers, Doerflinger, and Davidson (2006) using a food typically high in fats rather than sugars: rats were given potato chips that either were consistently a source of fats and calories (consistent group) or potato chips that were sometimes high-fat and sometimes low-fat (inconsistent group). The low-fat potato chips in this experiment used a non-caloric fat substitute to replace and mimic the taste and sensory characteristics of the chips' natural fat. Similar to the results found by Davidson & Swithers (2004), the study focusing on fat/calorie pairings also found that rats with unreliable food cues were impaired in their regulation of total calorie intake (Swithers, Doerflinger, & Davidson, 2006).

The cafeteria diet is one method that researchers use to study the effect of a modern diet on eating behavior. A cafeteria diet involves providing animals

with a large variety of human foods. The first cafeteria diet study was done by Sclafani and Springer (1976), and it found that rats fed a variety of "supermarket" foods were more likely to become obese than control rats in addition to being impaired at maintaining their increased weight. The cafeteria diet incorporates aspects of variety in sensory and nutrient composition, as well as aspects of a high-fat high-sugar diet and increased palatability (McCrory, Burke, & Roberts 2012). Animals are prone to favor variety in their diet, since a varied diet is more likely to include the many vitamins and nutrients needed by the body. However, variety in modern human diets may be more harmful than helpful.

Animal models in a laboratory are effective ways to study food-related behavior because animals exhibit the same basic motivations and food-related behaviors as humans, without many of the complicated behavioral, psychological, and ethical restrictions. Rats are especially appropriate models for food-related research because they share many similar attributes with humans, such as their generalist omnivore diets and the same basic motivations and taste preferences.

Researchers have found the cafeteria diet to have various effects on rats. Rats on a cafeteria diet often become overweight and possibly obese. One reason for this weight gain is that the variety of foods provided in a cafeteria diet allows rats to eat more food than control rats on a chow diet, due to the reduced likelihood of habituation to a single food (Louis-Sylvestre *et al.*, 1984). Even in humans, dietary variety is correlated with body fat, perhaps more strongly than the correlation between body fat and dietary fat (Yao *et al*., 2003). Cafeteria diets often include foods that are more calorically dense than rodent chow, which also

likely contributes to the occurrence of obesity in rats on the diet. These foods can be high in sugars and fats, which increase a rat's adiposity (Sclafani, 2004). Foods in a cafeteria diet are also more stimulating than standard chow because they have increased levels of rewarding orosensory properties such as taste, texture, and smell (Sclafani, 2004).

Beyond the physical reasons for weight gain on a cafeteria diet, there is also evidence that cafeteria diets cause psychological changes that induce increased food consumption. Rats kept on a cafeteria diet exhibit impaired sensory-specific satiety, meaning that they do not habituate to and stop eating a recently-consumed food as readily as a control rat (Reichelt, Morris, & Westbrook, 2014). As an animal consumes a food, typically the food becomes less palatable throughout the meal until the animal stops eating that food. Reichelt, Morris, and Westbrook (2014) found that rats that were allowed to drink one caloric flavored solution to satiety and then given the choice between the same solution and a new solution were less likely to prefer the new solution if they had been on a cafeteria diet. Thus, not only can rats on a cafeteria diet switch to eating a different food once sensory-specific satiety decreases their current consumption of one food, but also sensory-specific satiety occurs more slowly with experience on the cafeteria diet (Reichelt, Morris, & Westbrook, 2014).

As in simpler flavor-calorie reliability experiments, cafeteria diets also likely impair body weight homeostasis, which is normally regulated by caloric compensation in response to different foods (Prats, Monfar, Castella, Inglesias, & Alemany, 1989). A final behavioral change wrought by the cafeteria diet is a

change in rats' meal patterns. Cafeteria diet experienced rats demonstrate a tendency to consume many snacks throughout the day rather than a few larger meals (Martire *et al.* 2013; Rogers & Blundell 1985). Rogers & Blundell (1985) found that palatability and variety aspects of a cafeteria diet had distinguishable effects on feeding behaviors; palatability influences meal size, and variety changes the frequency of meals.

In past studies, cafeteria diets have typically consisted of a variety of human foods. Examples of some foods that have been included in cafeteria diets are bologna, cheerios, pineapple, and cookies (Perez, Fanizza, & Sclafani, 1999). These foods are atypical for what animals are evolved to eat as part of their wild diets. However, the condition to which cafeteria diet raised rats are compared is almost always a chow-only control group. While this is standard for rodents in laboratories, a diet consisting solely of rodent chow is not much closer than a processed cafeteria diet to a natural diet in the wild. A diet made up entirely of one food would not naturally occur in an animal's natural habitat because nutritionally complete foods like chow, which is strategically composed of the range of nutrients needed by rats in their diets, do not exist naturally in any one superfood. Until now, no studies have compared the effects of the unnatural processed cafeteria diet and chow-only diet with a cafeteria diet composed of a variety of natural foods. A diet consisting of various un-modified foods most closely mimics a natural diet that would be consumed by animals in the wild.

Another modern example of flavor-nutrient inconsistency is the widespread use of artificial sweeteners. Artificial sweeteners are chemical

compounds that taste sweet on the tongue yet contain little or no calories, meaning that they have no postingestive component (Yang, 2010). Despite the reduced calories, artificial sweeteners contribute to the high prevalence of extreme sweetness in the modern human diet. Humans and other animals have not evolved to regulate their diets in terms of the high levels of sweetness that are present in a large percentage of modern foods. Artificial and natural sweeteners are added to many foods that are not typically considered sweet or desired to be sweet, simply to increase the palatability. The presence of sweeteners in so many foods adds to the pharmacokinetic properties of a modern diet, which relates processed foods to drugs of abuse (Schulte, Avena, & Gearhardt, 2015).

Artificial sweeteners are commonly used in place of natural sweeteners in an effort to consume fewer calories. However, there is recent evidence that artificial sweeteners may have disruptive psychological consequences that conflict with dieting. First, there is evidence that artificial sweeteners alter the brain's response to sweetness; in humans, fMRIs have shown that the brain areas activated by sweet taste are different between diet soda drinkers and non-diet soda drinkers (Green & Murphy, 2012). Also in humans, habitually high consumers of artificial sweeteners are less responsive to sweet taste and its stimulating effects on appetite (Appleton & Blundell, 2007).

Typically, sweet flavors are predictive of the calorically dense postingestive effects of sugar. Artificial sweeteners disrupt the predictive value of sweet flavors by adding a flavor-nutrient relationship between sweet taste and no calories (Swithers & Davidson, 2008). Research in rats has shown that rats

exposed to unpredictive relationships between sweet taste and calories develop greater adiposity than rats given foods whose flavors are predictive of their calories (Swithers & Davidson, 2008). These results may be indicative of the inability of rats given confusing sweet flavors to regulate their energy homeostasis (Swithers, Martin, & Davidson, 2010). In general, artificial sweeteners can cause increased body weight and decreased caloric compensation, as compared to animals fed natural sweeteners (Swithers, Martin, & Davidson, 2010). This effect is found in humans in addition to rodents; one study found low-calorie sweeteners to be positively associated with humans' Body Mass Index (Miller & Perez, 2014). This correlation between artificial sweeteners and weight gain is another example of how decreasing the reliability of a flavor-nutrient relationship might disrupt new flavor-nutrient learning and thus impair body weight regulation and food intake (Swithers, 2013).

Animals are born with few innate taste preferences, which are mainly an unlearned preference for fats and sugars (Ackroff, Vigorito, & Sclafani, 1990). To survive, animals must learn about the flavors that they encounter in their environment. Animals learn to prefer certain flavors over others by classical (Pavlovian) conditioning, in which they experience and learn about flavors paired with different foods which have positive or negative postingestive consequences (Myers & Sclafani, 2006). In Pavlovian conditioning, an unconditioned stimulus (US) which naturally elicits a certain natural unconditioned response (UR) is continuously paired with a conditioned stimulus (CS). The conditioned stimulus gains significance as a result of its pairing with the US, and the CS eventually

elicits a new behavioral response called the conditioned response (CR). This process occurs with flavors and nutrients. Nutrients, such as sugars or fats, are unconditioned stimuli that stimulate chemosensors in the gut to sense the positive presence of nutrients (Sclafani & Ackroff, 2012). The sensing of nutrients is the UR. Postingestive sensing of the US also stimulates the dopamine reward system in the brain (Ackroff, Vigorito, & Sclafani, 1990). Flavors that are paired with nutritive foods are conditioned stimuli. After repeated pairing of a nutrient (US) and flavor (CS), the flavor itself is capable of eliciting increased intake and liking of a substance (CR). This is called flavor-nutrient conditioning.

Flavor-nutrient conditioning is an adaptive learning behavior in nature. Flavor-nutrient learning has long-lasting effects and the actual learning occurs quickly; rats can acquire a preference for a nutrient-paired flavor in as little as one trial (Myers, 2007). This allows animals to make connections between the flavors they experience orally and the satiating sensations that they sense postingestively. Learning the relationships between flavors and their postingestive consequences allows an animal to choose foods in the future that satisfy its body's needs. This process is particularly important for efficient foraging behaviors. Disruption of flavor-nutrient conditioning is harmful because without reliable understanding of which foods are calorically dense and satisfy certain nutrient requirements, an animal's diet and body can suffer from malnutrition and a deficit in certain nutrients.

A flavor-nutrient conditioning test is a common technique to evaluate how well an animal has learned the relationship between a particular solution and its

paired flavor. This test unconfounds the often-related effects of palatability and calories by keeping palatability constant and manipulating caloric density. Research shows that when given two differently-flavored solutions of equal palatability and different caloric content, typical rats will establish a conditioned preference for the higher calorie solution (Warwick & Weingarten, 1993). The two solutions' nutrient content serve as the unconditioned stimuli of Pavlovian conditioning, and the paired flavors are the conditioned stimuli.

Two main aspects of flavor-nutrient conditioning are preference and acceptance. Animals develop a preference for the CS+, which is the flavor that has been consistently paired with the more calorically-dense solution. Increased preference for a preferred solution causes increased consumption of that one flavor/solution relative to a less preferred solution. However, flavor-nutrient conditioning also involves increased acceptance. Acceptance describes how animals consume progressively larger quantities of solution over time due to their experience with the solution. Acceptance is not the same as preference; in acceptance, the absolute intake by an animal increases because the animal accepts the flavor more with time. Preference causes only a shift in the percentage of intake for two (or more) solutions being compared by the animal, without causing overall intake to increase (Myers & Sclafani, 2006). Preference and acceptance do not always have to increase simultaneously (Perez, Lucas, & Sclafani, 1998). Acceptance can increase intake of a flavor that was initially disliked by an animal, and preference can cause the animal to choose that flavor instead of another flavor (Perez, Lucas, & Sclafani, 1998).

Some previous research has investigated whether flavor-nutrient learning is affected by a modern diet. Perez, Fanizza, and Sclafani (1999) conducted flavor-nutrient conditioning with rats that were fed a cafeteria diet that consisted of four possible food combinations. Flavor-nutrient conditioning in this instance involved intragastric infusions of Polycose or water, which were paired with orally ingested, equally-preferred solutions of sodium saccharin and water with either grape or cherry flavor. No difference in flavor-nutrient learning was found between rats on the cafeteria diet and control rats (Perez, Fanizza, & Sclafani, 1999). However, we believe that this study was limited in its methodology and results. The four food combinations comprising the cafeteria diet were internally consistent; for example, bologna, green peas, oatmeal cookies, yogurt, and chow were always given together (Perez, Fanizza, & Sclafani, 1999). This cafeteria diet did not offer as much variety or difficult flavor-nutrient relationships as possible, and so we believe that different results could have been obtained with a more complicated and varied cafeteria diet, paired with more extensive experience on the diet. Additionally, Perez, Fanizza, and Sclafani (1999) did not account for the effects of variety separately from the high-fat high-sugar (HFHS) or flavor-nutrient confusion effects of a processed cafeteria diet. The present study was designed to separately examine some of these effects by incorporating a natural foods cafeteria diet.

The current study will contribute to the known research on cafeteria diets and the effect on flavor-nutrient learning and preferences. While the cafeteria diet is an intriguing way to study basic animal behavior in response to modified

human diets, interpretation can be problematic. The effects of cafeteria diets can be attributed to several different factors, which include variety, high-fat high-sugar components, palatability, and flavor confusion. This study was designed to be one step towards unconfounding several of these aspects. Traditional cafeteria diets combine a variety of human-typical foods, such as spaghetti, candy, and cheese crackers. One type of cafeteria diet that has never been published is one that maintains the traditional cafeteria diet's variety without the increased high-fat high-sugar and added palatability. Our research included a traditional human foods cafeteria diet and also incorporated a new method with a more consistent pattern of flavor-nutrient relationships: the natural foods cafeteria diet. Despite the lack of significant results found by Perez, Fanizza, and Sclafani (1999) between cafeteria and control rats on intragastric flavor-nutrient conditioning, we believed that our more expansive variety, more inconsistent, and more extensively-given cafeteria diet might produce different results.

Rats were used in this study, as in many other appetite and learning studies, because they are intelligent animals that exhibit many of the same behavioral and psychological responses as humans. Rats are generalist omnivores, and they are good models of appetite.

In the current study, 36 rats were given experience for 3 months on a processed foods cafeteria diet, natural foods cafeteria diet, or chow-only control diet. They were then tested in multiple ways on Flavor-Nutrient Conditioning to analyze whether a history of variety or flavor confusion impaired new flavor-nutrient learning. This testing was followed by Progressive Ratio lever-pressing

for a sucrose reward to determine if the groups were differently motivated to work for sucrose after their experience on the diets. Finally, after a general trend in decreased drinking of sweet solutions by cafeteria diet rats was noted, all rats underwent lick microstructure analysis to examine their perceived palatability of a sweet solution.

Different experiences with food can shape an animal's perceived value of a certain food. Two components that affect reward value are motivation and palatability. These are plastic characteristics that can change with time and experience. An animal's motivation for a food reward is measured by how much effort it is willing to expend to receive the reward. Researchers have studied this question by using a progressive ratio lever-pressing for reward test. In a progressive ratio operant schedule, rats must press a lever to receive a food or sucrose pellet, and each subsequent pellet requires an increased number of lever-presses. The data analyzed from this test is each rat's breakpoint, which is the number of lever-presses necessary for the final reward that a rat attained. Rats show near-linear increases in break point as a function of sucrose concentration, regardless of their level of satiety (Sclafani & Ackroff, 2003). Assuming that rats' motivation to work for sucrose is a similarly linear function as a result of concentration, this research establishes progressive ratio lever-pressing as a reliable measure of food reward (Sclafani & Ackroff, 2003). The relationship between reward concentration and breakpoint is likely based on gustatory liking of the reward rather than postingestive caloric reward, because other research has

found the similar breakpoint vs. concentration ratios for both sucrose and the non-caloric sweetener saccharin (Reilly, 1999).

Progressive ratio lever-pressing has been used to compare the motivation to obtain sucrose pellets between rats on a chow (control) diet and those on a high-fat high-sugar (HFHS) diet. Rats on a HFHS diet demonstrated significantly higher counts of active lever presses to obtain sucrose pellets (la Fleur *et al.*, 2007). This study demonstrates that experience on an obesogenic diet, such as a HFHS diet, can modify rats' motivation to work for a sucrose reward by changing its reward value according to a rat. In contrast to the results found by la Fleur *et al.* (2007) that HFHS diets have increased motivation to work for sucrose, other research has found that experience on a high-fat diet decreases rats' motivation for sucrose (Tracy, Wee, Hazeltine, & Carter, 2015). Although these studies contradict each other, they show that experience on a manipulated diet can alter a rats' motivation for sucrose, which suggests that a cafeteria diet might also influence rats' behavior in a progressive ratio lever-pressing task for a sugar reward.

Another approach for analyzing an animal's perceived food reward value uses lick microstructure analysis. This method is used to analyze a stimulus's palatability. Palatability can be affected by flavor-nutrient conditioning or flavor-flavor conditioning, and it can also be affected by repeated exposure to a stimulus (Liem & de Graaf, 2004). Lick microstructure analysis is based on the fact that when rats lick a solution, licks are grouped into "clusters." Clusters are separated by brief periods of non-licking. Lick microstructure analysis looks at the number

of licks, the number of lick clusters, and the size of lick clusters within a session. Interlick interval can also be evaluated. Total intake, which is measured by the number of licks, often decreases with increasing concentration of a solution (Davis & Smith, 1992). This is due to the earlier onset of satiety as a result of more concentrated solutions. Lick cluster size typically increases with the concentration of sweetness, and thus cluster size is used as a measure of palatability since sweetness is positively correlated with palatability (Dwyer, 2008). The size of a lick cluster is probably regulated by neural processes influenced by the food stimulus's effect on the gustatory system (Spector, Klumpp, & Kaplan, 1998). Regardless of what length of time is defined as the inter-cluster interval, increasing sucrose concentration reliably increases cluster size (Spector, Klumpp, & Kaplan, 1998). The analysis of cluster size can thus be applied to novel solutions by understanding larger cluster sizes to be indicative of more palatable solutions.

In summary, the current study involved manipulating rats' experience with foods with either a processed or natural foods cafeteria diet. After extensive exposure to the cafeteria diets, rats were tested on their ability to form learn new flavor-nutrient relationships. They were also tested on a progressive-ratio operant task and analyzed using lick microstructure to determine whether history of being on a processed or natural foods cafeteria diet manipulated the rats' perceived reward value of sugar.

Methods

Subjects & Housing

36 female Sprague-Dawley rats were used as subjects in this study. Subjects were bred and born in the laboratory at Bucknell University in Lewisburg, PA. Once weaned, subjects were pair-housed, which was with a littermate whenever possible. Rats were housed in plastic cages lined with bedding and topped with ventilated metal lids. Rats had access to water and rat chow *ad libitum*, except when experimental protocol required otherwise. Subjects lived in a room with a 12:12 light:dark cycle, with lights on at 8am every morning.

Experimental Conditions & Diets

Rats were assigned to one of three dietary conditions: processed foods cafeteria diet (PF), natural foods cafeteria diet (NF), or control (CON). Condition assignments were done by litter and by weight, such that two rats from each litter were assigned to each condition, and assignment from each litter was balanced by body weight. All rats had *ad libitum* access to rat chow except when noted otherwise, and PF and NF rats received additional foods daily according to their experimental condition. All rats started their respective diets when they were two months old. The diets were in place from July to October 2014, during which time rats were pair-housed with a littermate assigned to the same condition.

"Processed foods" in this experiment were foods that were substantially modified beyond natural ingredients, incorporating added sugars, added fats, and manipulated sensory characteristics such as artificial flavors. On any one day, the

PF group usually received one sweet item and one nonsweet item. Examples of processed foods included raspberry granola, honey buns, pretzels, and baked beans. In contrast, "natural foods" designated foods that were minimally processed by humans, and included no manipulated flavors, added sugars, or added fats. "Natural foods" included many fruits, vegetables, and grains; some examples are pears, kale, and teff. See Appendix for a complete list of foods, rations, and nutritional information.

Initially, the PF and NF diets involved each cage receiving two novel foods per day. Each food was given individually in a removable plastic cup attached to the inside of the cage. For each food item, a pre-determined ration was established by weight. Food rations were chosen to approximate equivalent volumes, so that PF & NF groups were given equal volumes of foods per day. Every afternoon approximately 24 hours after the previous feeding, a researcher weighed the leftover amount of each food from the day before, and then refilled the cups with new foods. Novel foods were given every day until 78 foods had been given to the PF and the NF groups. At this point, foods were cycled through again but in a random order so that food pairings were never consistent. After several weeks on the experimental diets, the protocol changed to each cage receiving 3 foods per day. Of these three, two were familiar foods that had already been received once or twice before, and one food was novel in order to give subjects experience with an even greater variety of foods. In total, each group was exposed to about 90 different foods. Cafeteria diets continued until rats had been

on their experimental diets for a total of three months, at which point all rats were returned to a chow-only diet before behavioral experiments started.

Flavor-Nutrient Conditioning 1: Learned Preference

Flavor-nutrient conditioning is a behavioral test in which animals are familiarized with two differently flavored solutions of significantly different caloric densities, and then preference between the two flavors is tested to determine whether the animal has learned to prefer the flavor paired with the more calories. To avoid a preference based on hedonic value rather than caloric density, the experiment necessitated two solutions with equivalent palatability but different postingestive nutritional consequences in the gut. Two solutions that are about equally palatable to rats are a solution of moderate glucose concentration and a solution combining a low concentration of glucose with a low concentration of saccharin. These solutions both taste sweet to a rat, but the one high in glucose has a much higher caloric density than the glucose/saccharin mixture.

Pilot

A pilot experiment with ten naive female rats was conducted to determine exact concentrations of glucose and glucose/saccharin solutions that our rats would consume equally based on hedonic value. Based on a similar procedure done by Warwick & Weingarten (1994), one solution was set at a concentration of 1% glucose/ 0.125% saccharin. To determine the glucose concentration of the second solution, we tested the pilot rats with glucose solution concentrations of 2%, 4%, 6%, and 8% in a two-bottle test where the two solutions were always a

glucose solution and the glucose/saccharin mixture. Based on average intake, the 1% glucose/ 0.125% saccharin solution was mathematically determined to be equally palatable to a solution of 6.5% glucose.

FNC1 Two-Bottle Preference Test

Before Flavor-Nutrient Conditioning could begin, rats were separated into individual cages to allow precise intake measurements, and they were put on a restricted chow-only feeding schedule. Rats were provided chow every evening after the onset of the dark period once any experiments were finished for the night, and leftover chow was removed every morning soon after the onset of the light period. This restricted feeding schedule was established so that rats would be hungry and ready to eat every evening around the dark period onset, which would induce increased rates of drinking for the subsequent experiment.

All stages of Flavor-Nutrient Conditioning were conducted in the rats' home cages. Bottles of solution were always weighed before and after sessions to calculate intake. The first stage of FNC was two days of familiarization to habituate the rats to drinking from a bottle immediately after it was placed on their cage lid. On familiarization days, each rat received a bottle containing 20 mL of 1% glucose/0.125% saccharin solution for 1 hour.

After the familiarization days, the next stage of FNC was one-bottle exposures to flavor-paired glucose and glucose/saccharin solutions. Over the four days of one-bottle exposures, rats alternated getting a bottle 15 mL of 6.5% glucose solution one day and 0.1% glucose/ 0.125% saccharin solution on the other day. Rats had access to their bottle for 2 hours beginning immediately after

the lights went out at 8pm. For each rat, the solutions were consistently paired with the Kool-Aid flavors of orange and lemon/lime. Across rats, the flavor/solution pairings were counterbalanced such that the glucose solution flavor (CS+) was orange for some rats and lemon/lime for other rats, and each rat's glucose/saccharin mixture flavor (CS-) was the opposite. Orange and lemon/lime were chosen because no rats had received any citrus flavors during their experimental diets, and so all subjects were equally inexperienced with orange and lemon/lime flavors. Additionally, in the prior research of this lab and others using this protocol, it has been determined that naive rats generally have no preference between orange and lemon/lime flavors.

The culmination of Flavor-Nutrient Conditioning was the two-bottle test, which assessed rats' preference for the CS+ flavor and CS- flavor. For the two-bottle test, each rat had access to two bottles of 40 mL of 1% glucose/0.125% saccharin solution. One bottle contained solution flavored with orange, and the other bottle's solution was flavored with lemon/lime. Although both flavors were presented in glucose/saccharin solution on the test days, one of the flavors had been consistently paired with the calorically dense glucose solution during the previous one-bottle exposures. Thus, this measured conditioned change in flavor preference. The two-bottle test was done on two consecutive days. Relative positions of the two flavors were counterbalanced across and within test days to eliminate a side-preference effect. Intakes of each flavor was averaged across the two two-bottle test days to produce average intakes and preferences of the flavor

for each rat that was previously paired with glucose solution (CS+) and for the flavor previously paired with glucose/saccharin solution (CS-).

Flavor-Nutrient Conditioning 2: Learned Acceptance

After the first round of Flavor-Nutrient Conditioning was completed, a new experiment called here Flavor-Nutrient Conditioning 2 (FNC2) was conducted to experimentally determine how much of the rats' consumption of the glucose-paired flavor in Flavor-Nutrient Conditioning was due to learning about the flavor and its association with glucose, and how much of the consumption was due to inherent liking for the mixture in which the flavor was presented for the two-bottle test.

For FNC2, rats continued on the restricted-chow schedule, in which an abundance of chow was provided at night and leftovers were removed in the morning. Rats remained singly housed for the purpose of measuring exact intakes per rat.

New flavors were required for FNC2, since rats had previous, confounding experience with the flavors used in FNC1. A pilot experiment with 10 naive female rats was conducted to test which of several flavors were approximately equally palatable. These two flavors were determined to be coffee and butter, from extract (McCormick brand), with which the rats had no prior experience in tests or in diets.

For the first two days of FNC2, rats received a bottle of 40mL of unflavored 1% glucose/ 0.125% saccharin solution for two hours, beginning

immediately after the lights went out in the housing room at 8pm. Intake of the solution was measured to determine each rat's baseline intake of the mixture solution.

The next stage in FNC2 was one-bottle training sessions, where rats received one bottle of either the glucose or the mixture solution each evening. Like the previous experiment, the flavor/solution pairs were balanced such that glucose solution was paired with coffee flavor for some rats and butter flavor for other rats, and then for each rat the mixture solution was presented in the opposite flavor. One-bottle training sessions lasted for 6 days, and the order of solutions over the six days was [CS+, CS-, CS-, CS+, CS+, CS-]. The pairing of solutions with flavors was equally balanced across all 36 rats, i.e. for half of the rats the glucose solution was paired with coffee and mixture with butter flavor, and for the other half the glucose solution was paired with butter and the mixture with coffee flavor. One-bottle training ran for two hours at the onset of the dark period, and each bottle contained 12 mL of solution. Total possible intake was limited to 12 mL based on the minimum intake of solution during the baseline tests, so that no rat could drink a significantly higher amount of any solution and gain more experience with it.

In order to measure what rats learned in the training phase, the ultimate FNC2 test involved 4 test days. For the first three test days, rats received one bottle each of 40 mL of glucose/saccharin solution for 2 hours at the onset of the dark period. The bottle contained either unflavored mixture, butter flavored mixture, or coffee flavored mixture. The order of these three different flavors was

counterbalanced amongst the rats across the three days. Each day, the bottles were weighed to determine intake. After these three days, calculations determined the percentage increase that the rat drank of its CS- flavored mixture relative to the amount of the unflavored mixture, as well as the percent increase of CS+ flavored mixture relative to the unflavored mixture. The CS- percent increase and CS+ percent increase were then averaged for each experimental group (PF, NF, and CON) and compared. The purpose of this experiment was to determine whether the CS+ flavor stimulated intake over and above inherent palatability of the glucose/saccharin mixture itself.

Finally, the fourth test day was a two-bottle test similar to the one done in FNC1. Each rat had access to 2 bottles of 40 mL of glucose/saccharin mixture, one butter-flavored and one coffee-flavored, and intakes were measured.

Ad Lib Sweet Consumption

One brief experiment was to measure each group's intake of a sweet solution provided *ad libitum.* Rats in individual cages received a bottle of unlimited 2% sucrose/ 0.2% saccharin solution. Two of these sessions were conducted, each lasting 2 hours. Bottles were weighed before and after sessions to calculate average intake.

Progressive Ratio Lever-Pressing For Sucrose Reward

Progressive Ratio lever-pressing is a test that measures an animal's motivation to work for a reward, based on how many times they will press a lever

to receive that reward when the requirement progressively increases. To motivate subjects to learn to lever-press, the rats were once more put on a restricted chow-only diet where rats received a chow ration every afternoon. Before this experiment, all subjects were weighed to obtain their free-feeding body weights. Each rat was assigned an individualized daily chow ration by weight based on the equation [4.5*(BW/100)-1], where BW is body weight. The goal of this food deprivation was to bring the rats' body weights down to between 90-95% of their free-feeding body weights. Once this feeding regimen started, rats were weighed every 2-3 days and their chow portions were adjusted accordingly to keep body weights within the targeted range.

Each stage of this experiment took place in an operant box, which is a small box with a door on one side and a trough on one wall beside a retractable metal lever. At the top of the operant box is a house light, which remained illuminated for the duration of a rat's session in the box. The trough is connected to an automated pellet dispenser, which was filled with 45 mg sucrose pellets**.** There is a light inside the trough, which illuminated when a pellet was dropped into it. The entrance of the trough is spanned by a small infrared beam whose path is disrupted when a rat enters its head in the trough, alerting the computer. The metal lever is connected to a contact sensor that alerts the computer when the rat makes contact with the lever; presses and touches to the lever are registered separately. Since only 4 rats could be in the operant boxes at a time, subjects were randomly assigned to 9 squads. The testing order of these squads was randomized

for each day so that time of day was balanced across groups and could not consistently bias rats towards better or worse performance.

Before conducting the actual progressive ratio lever-pressing test, subjects underwent a lengthy training protocol to shape them to press a lever for a reward. Each time a rat met the reward requirement for their current stage of the experiment, a sucrose pellet was released into the trough, and the light within the trough was illuminated. The rat's actions would only count towards a new pellet once the rat had stuck its nose into the trough to retrieve the previous pellet. Trials in beginning training stages lasted for thirty minutes each**.** The success of subjects on each stage of training was monitored, and if a rat did very poorly on a new stage, she was sometimes moved back a stage or put on a "remedial" schedule to bring all subjects up to equivalent baseline lever-pressing proficiency.

Shaping began with magazine training, which was a 30 minute session for the rats to associate illumination of the pellet trough with pellet delivery. Once the rat's head entered the magazine, a pellet dropped and the light in the food trough was illuminated until the pellet was retrieved. The next step was Touch training, in which the lever was periodically inserted and retracted in the operant box and a rat received a pellet if she touched the lever. Every 60-90 second ITI, the lever inserted into the operant box. If the rat touched the lever, she received a sucrose pellet immediately. If 15 seconds passed without the rat touching the lever, a sucrose pellet dropped anyways and the lever retracted until the next lever insertion.

After rats would reliably touch the lever and retrieve the pellets, the next stage of training was “Press” training, for which touching the lever was no longer sufficient. In Press training, a rat had to fully depress the lever to receive a sucrose pellet. The lever remained inserted in the box throughout the trial except if a rat did not lever-press within 15 seconds, at which point the lever briefly retracted and then re-inserted itself.

Once the rats could reliably press the lever and retrieve their reward, the next stage in training was four sessions of continuous reinforcement (CRF). On the CRF schedule, the lever inserted into the box at the beginning of the trial and stayed inserted for the duration of the session. Each lever press garnered one sucrose pellet. The rat was free to press the lever and receive its pellets for a total time of either 30 minutes or until the rat had received 150 pellets, whichever came first.

The next stage after CRF was Fixed Ratio (FR) lever-pressing, in which the rat had to press the lever a fixed number of times (more than one) per sucrose pellet. Rats first underwent two sessions of FR-3 trials, in which three lever presses had to be registered before a sucrose pellet was dropped. One session of FR-5 trials was next, in which a rat was required to execute five lever presses per pellet. Finally, subjects were ready to proceed to Progressive Ratio lever-pressing.

On a progressive ratio (PR) lever-pressing schedule, rats have to press the lever an increasing number of times within each trial in order to receive a sugar pellet. A formula created to determine the number of presses per pellet in this schedule, which is often used in drug tests, is [5e^(R*0.2)]-5 (Richardson &

Roberts, 1996). Thus, the rat must press the lever once for the first pellet, twice for the second pellet, four times for the third, and so on. The number of lever-presses necessary to gain the first twenty pellets are as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268. Trials ended after 90 minutes or after a rat had not earned a pellet for 15 minutes. Subjects underwent progressive ratio trials once every other day for a total of three times each.

The ultimate goal of conducting Progressive-ratio lever pressing was to measure each individual rat's breakpoint. The breakpoint in this experiment was the number of times that a rat was willing to lever-press for a sugar pellet; thus, it was the number of lever-presses required for the last pellet that the rat received in a trial.

Lick Microstructure

Lick Microstructure is an experimental method to analyze how palatable a solution is perceived to be by an individual animal. Animals provided with some solution are put in individualized lick boxes connected to a computer that registers the exact timing of each lick. Then computer software can be used to compare total number of licks, total number of lick clusters, and cluster size across groups and across different concentrations of solution.

Subjects were pair-housed and put back on their respective PF, NF, and CON diets for three weeks. To give them more experience with the foods they received, cafeteria food rations during this time were 120% of the previous rations. After 3 weeks back on the cafeteria diets, subjects were weighed for free-feeding

body weights and then each cage (pair of rats) was assigned an individualized daily chow ration to bring subjects down to within 90-95% of free-feeding body weights. Rats were fed chow rations in the late afternoon every day.

After several days on the restricted chow schedule, rats were first re-familiarized drinking out of a bottle immediately after the bottle appears. For two days, each pair of rats was given access to two bottles of 1% glucose/ 0.125% saccharin solution. Next, rats were familiarized with the lick microstructure apparatus and drinking procedure.

The apparatus used for lick microstructure is a cylindrical enclosure with an opening on one wall where bottles can be mechanically inserted or retracted for the sipper attached sipper tube to be within reach of the rat in the box. The floor of the apparatus is a metal grid with a slight electrical current (in the nano-ampere range, far below the threshold of what the rat could feel) running through it, and the apparatus is connected to a computer. Every time that a rat licks the metal sipper tube of the bottle in front of their enclosure, the electrical circuit is completed and the computer registers the precise time and number of the lick.

Rats underwent two familiarization sessions with the lick microstructure apparatus and procedure. During these sessions, rats spent thirty minutes in the lick boxes with access to a bottle of 1% glucose/ 0.125% saccharin.

For the actual lick microstructure sessions, rats were tested with three different solutions: a low, medium, and high concentration of sucrose (5%, 10%, and 30% sucrose). Rats were tested twice with each of the three concentrations, which were balanced across rats and across sessions. Each lick microstructure

session lasted thirty minutes, where a rat in each lick box had access to an unlimited amount of sucrose solution in the adjacent bottle. Data collection consisted of the number of licks by each rat as well as the timing of each lick. A software program later converted that data into the number of lick clusters per rat and the average size of the rat's clusters. The six sessions in total were conducted over the span of two weeks, and always occurred in the mid-afternoon. Subjects were necessarily tested in four groups to accommodate the number of lick boxes, with the testing groups balanced by experimental group.

Results

Body Weights

After the three initial months of dietary conditions were finished and before the first test began, PF rats had significantly higher body weights than CON rats, and the weight of NF rats were intermediate between PF and CON rats [$F(2,33) = 0.435$, $p<.05$] (Figure 1). Throughout the course of the behavioral experiments once rats were all on chow-only diets, the weight difference between groups decreased until it was no longer statistically significant.

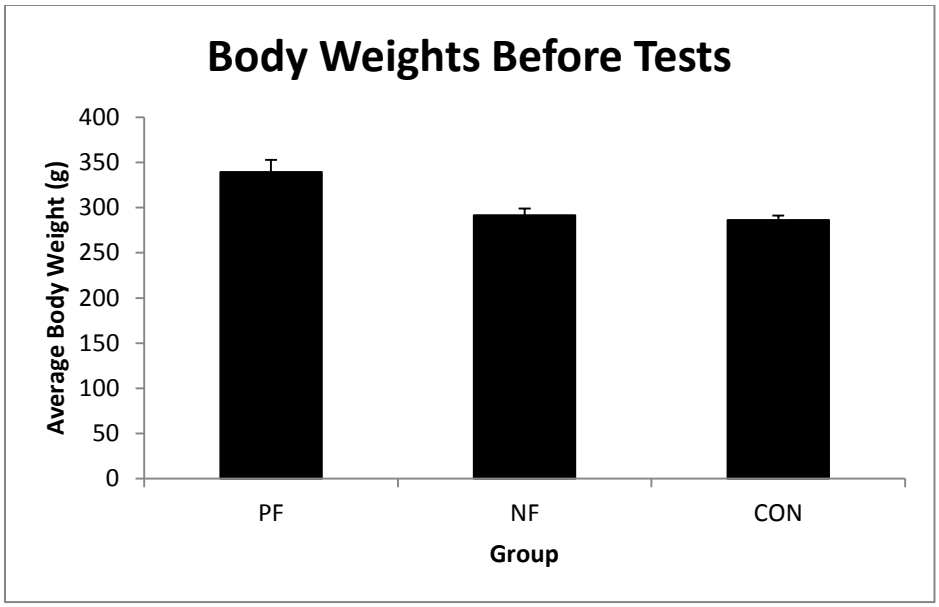

Figure 1: Body weights of PF, NF, and CON rats after three months on their experimental diets and before the first behavioral test was conducted.

Flavor-Nutrient Conditioning 1: Preference

In Flavor-Nutrient Conditioning 1, rats were given a two-bottle choice test between the CS+ flavor and CS- flavor after extensive experience with those flavors. Intakes of the two flavors were analyzed across groups in several ways. All rats preferred the CS+ flavor over the CS- flavor. A one-way ANOVA comparing the percentage of CS+ flavor preference in the two-bottle test revealed that CON, PF, and NF rats all had significantly higher intakes of, and thus preferred, the CS+ flavor over the CS- flavor [$F(2,33) = 1.882$, $p>.05$]. Contrast tests showed no significant difference between any two groups.

In the two-bottle choice test between the CS+ flavor and the CS- flavor, both presented in glucose/saccharin mixture, all groups showed significant differentiation between the two flavors [main effect of flavor, $F(1,33) = 192.76$,

p<.001], which was revealed by a 3 (Group) x 2 (Flavor) ANOVA. As seen in Figure 2, CS+ intake was significantly higher than CS- intake, which indicates that the rats learned an overall preference for the CS+ flavor. There was also a significant difference in overall intake between groups, whereby CON rats had the highest overall intake, followed by NF rats and then PF rats. [main effect of group, $F(2,33) = 5.569$, $p<.05$]. Finally, rats in different groups showed significantly different relationships between CS+ intake and CS- intake, indicating that CON rats learned better than NF or PF rats [flavor x group interaction, $F(2,33) = 7.966$, $p<.05$].

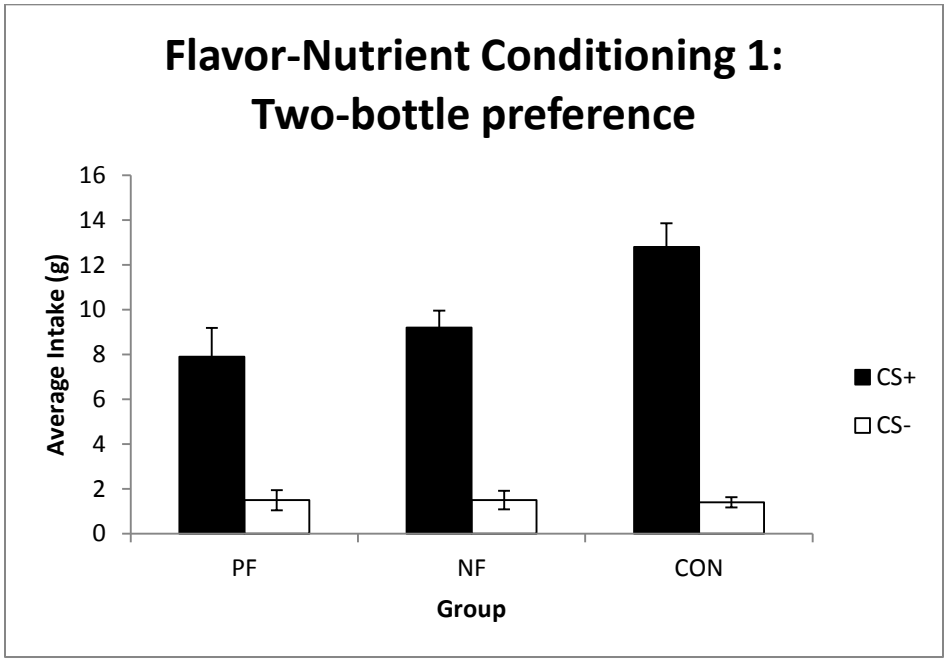

Figure 2: Intake of CS+ flavored and CS- flavored 1% glucose/0.125% saccharin solution in the two-bottle preference test of Flavor-Nutrient Conditioning 1

Flavor-Nutrient Conditioning 2: Acceptance

Two-bottle preference test

Flavor-Nutrient Conditioning 2 was designed to take rats' acceptance of CS flavors into consideration, in addition to preference. FNC2 culminated in two tests: a two-bottle choice test and a series of one-bottle intake tests. The two-bottle choice test was functionally identical to that of FNC1. In the two-bottle test for this second round of Flavor-Nutrient Conditioning, a one-way ANOVA showed that there was no significant difference in percent CS+ preference between groups [$F(2,33) = 2.282$, $p>.05$]. A 3 (Group) x 2 (Flavor) repeated measures ANOVA was then conducted on the two-bottle test between CS+ and CS- flavors, which showed that all rats drank significantly higher amounts of the CS+ flavor over the CS- flavor overall [main effect of flavor, $F(1,33) = 28.514$, $p<.001$]. This effect can be seen in Figure 3. Total intake did not differ between groups [main effect of group, $F(2,33) = 1.513$, $p>.05$]. However, the relationship between group and CS intake did not differ significantly across groups, which indicates that all rats learned equally [flavor x group interaction, $F(2,33) = .712$, $p>.05$].

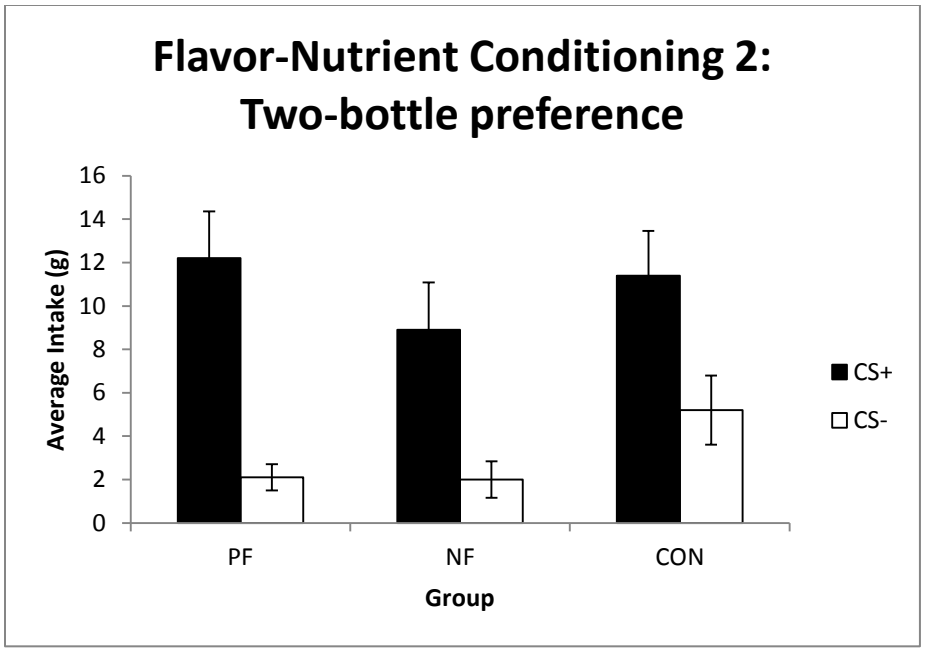

Figure 3: Intake of CS+ flavored and CS- flavored 1% glucose/0.125% saccharin solution in the two-bottle preference test of Flavor-Nutrient Conditioning 2

One-bottle acceptance tests

The main test of Flavor-Nutrient Conditioning 2 was the comparison of intakes between one-bottle tests of Unflavored, CS+, and CS- flavored glucose/saccharin solution. A 3(Group) x 3(Flavor) repeated measures ANOVA on the solution intakes across groups showed a significant effect of flavor, which confirms that the rats treated the three flavored solutions differently by consuming different amounts of different flavors [main effect of flavor, $F(2,66) = 19.403$, $p<.001$]. This can be seen in Figure 4. There was also a significantly different overall intake of solution between groups, in which CON rats had the largest overall fluid intake, followed by PF rats and then NF rats [main effect of group, $F(2, 33) = 6.336$, $p<.05$]. However, this ANOVA revealed no significant

interaction between flavor and condition, which would confirm the findings from the two-bottle test of FNC2 that all groups learned flavor-nutrient relationships equally well [flavor x group interaction, $F(4, 66) = .831$, $p>.05$].

To further examine patterns of one-bottle acceptance between groups, we conducted three separate one-way ANOVAs comparing the intake of Unflavored, CS+, and CS- flavored glucose/saccharin mixture within each individual group. In the control group, there was no significant difference in consumption of the three solutions [$F(2,22) = 2.437$, $p>.05$]. In the NF group, there was a significant effect of flavor [$F(2,22) = 10.447$, $p<.05$]. A contrast test showed that NF rats drank a significantly higher amount of CS+ solution than Unflavored solution [$F(1,11) = 24.431$, $p<.01$]. Albeit weaker, there was also a significant difference between CS- intake and the lower unflavored intake in NF rats [$F(1,11) = 6.205$, $p<.05$], meaning that they treated the CS- flavor as better than Unflavored, even though both flavors were previously paired with the nutritionally identical glucose/saccharin solution. The PF group also demonstrated a significant differentiation between flavors in their intake of the three solutions [$F(2,22) = 13.112$, $p<.001$]. Similarly to the NF group, the PF group consumed significantly higher amounts of the CS+ flavored solution than the Unflavored solution [$F(1,11) = 19.722$, $p<.05$]. Unlike the NF group, the PF group treated the CS- and Unflavored solutions equivalently, with no significant difference between intake of these two flavors [$F(1,11) = .422$, $p>.05$].

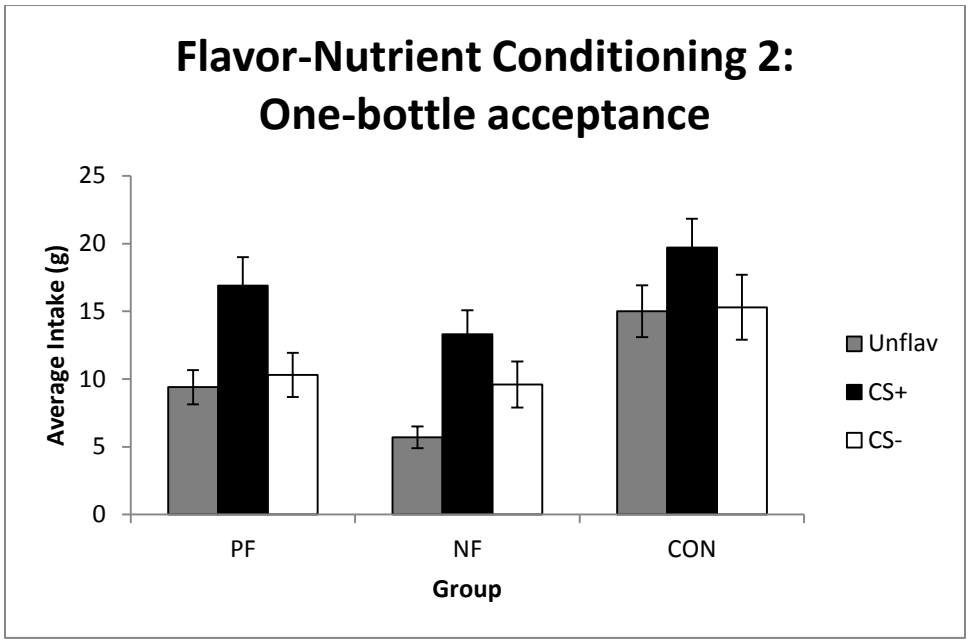

Figure 4: Intake of Unflavored, CS+ flavored, and CS- flavored 1% glucose/ 0.125% saccharin solution in the one-bottle acceptance tests of Flavor-Nutrient Conditioning 2

Ad lib Sweet Consumption

In this sweet consumption paradigm, rats were given *ad libitum* access to a sweet yet not calorically dense 2% sucrose/ 0.2% saccharin solution. The goal was to analyze whether CON, NF, and PF rats drank significantly different quantities of sweet-tasting solutions. Groups drank significantly different amounts of the sucrose/saccharin solution [$F(2,33) = 6.314$, $p<.05$]. A contrast test showed that the NF group ($p<.05$) and the PF group ($p<.05$) both drank significantly less of the sweet solution than the CON group.

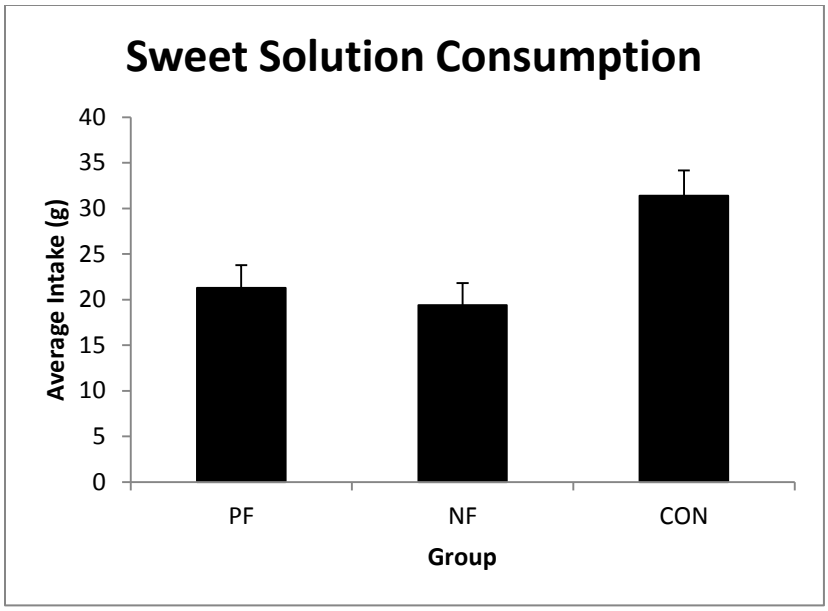

Figure 5: Intake of 2% sucrose/0.2% saccharin in the Ad lib Sweet Consumption paradigm

Progressive Ratio Lever-Pressing For Sucrose Reward

The Progressive Ratio lever-pressing test analyzed how motivated rats were to work for sucrose by measuring rats' breakpoint in terms of the number of pellets obtained, total lever presses, and total time length per session for each rat. A one-way ANOVA revealed that between groups there was no significant difference in the number of pellets obtained (Figure 6) [$F(2, 32) = .177$, $p>.05$]. There was also no significant difference between groups in the number of total lever presses during a session (Figure 7) [$F(2,32) = .075$, $p>.05$]. Finally, there was no significant difference between groups in the average length of time of a rat's PR session (Figure 8) [$F(2,32) = .974$, $p>.05$]. Contrast tests showed no significant differences between any two individual groups in any measure of PR

breakpoint. Results from Progressive Ratio testing indicated that rats with a history of different diets are not differently motivated to work for sucrose.

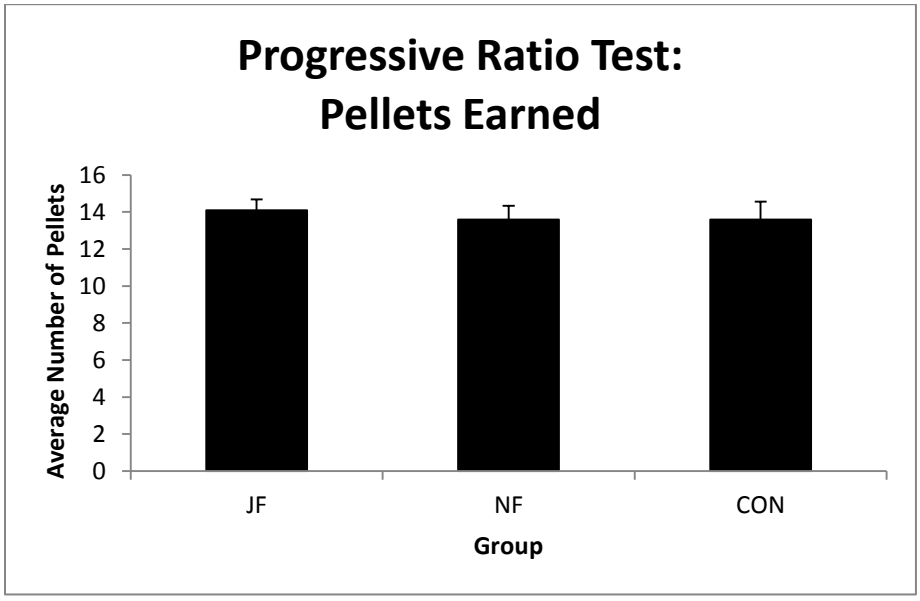

Figure 6: Total number of pellets earned across groups on a Progressive Ratio schedule

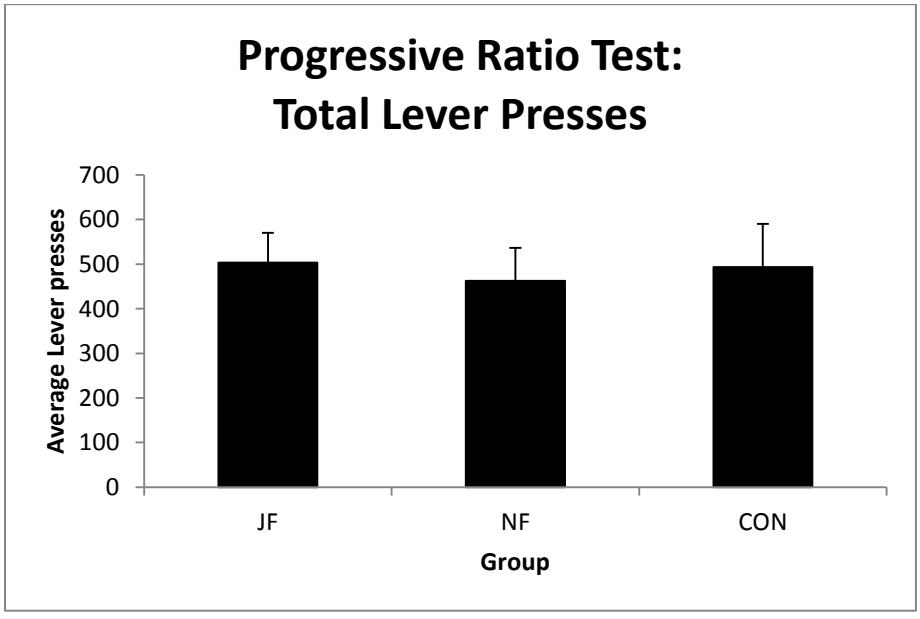

Figure 7: Total lever presses per session across groups on a PR schedule

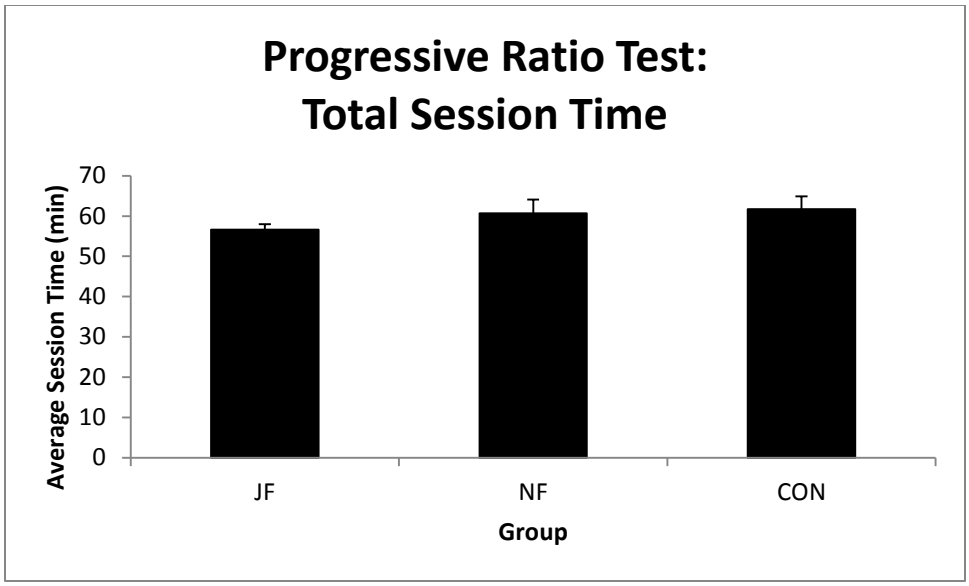

Figure 8: Average session time across groups on a PR schedule

Lick Microstructure

In this test, rats in lick boxes were given access to a bottle of sucrose. A computer attached to the apparatus measured total licks, average lick cluster size, and number of lick clusters per session. All subjects were tested with a low (5%), medium (10%), and high (30%) concentration of sucrose. The goal of Lick Microstructure was to analyze whether rats in different groups found different concentrations of sucrose more palatable.

A repeated measures ANOVA on total licks using the Greenhouse-Geisser analysis showed a significant effect of sucrose concentration on total licks [main effect of concentration, $F(1.496, 47.864) = 18.569$, $p<.001$]. There were less licks at higher sucrose concentrations because more concentrated solutions are more satiating (Figure 9). There was a significant effect of group on total licks [main effect of group, $F(2,32) = 4.805$, $p<.05$], where CON rats had the highest number

of licks, followed by NF rats, and then PF rats licked the least frequently overall. There was no significant difference in the pattern of total licks due to sucrose concentration across groups [concentration x group interaction, $F(2.992, 47.864) = 1.1$, $p>.05$].

A repeated measures ANOVA on the number of lick clusters per session using Greenhouse-Geisser analysis showed a significant effect of sucrose concentration on number of lick clusters for all rats, where for all rats the number of lick clusters decreased as sucrose concentration increased [main effect of concentration, $F(1.452, 46.453) = 41.771$, $p<.001$]. This effect is seen in Figure 10. Across groups, there was no significant difference in number of lick clusters [main effect of group, $F(2,32) = 2.174$, $p>.05$]. Finally, there was no significant difference between groups in the relationship between sucrose concentration and number of lick clusters [lick clusters x group interaction, $F(2.903, 46.453) = 2.024$, $p>.05$]

A repeated measures ANOVA on cluster size revealed that cluster size increased significantly with increased sucrose concentration (Figure 11) [main effect of concentration, $F(2, 64) = 27.306$, $p<.001$]. Cluster size did not differ significantly across groups [main effect of group, $F(2,32) = 2.727$, $p>.05$]. Interestingly, there was a significant difference across groups in the relationship between sucrose concentration and cluster size [cluster size x group interaction, $F(4, 64) = 2.785$, $p<.05$].

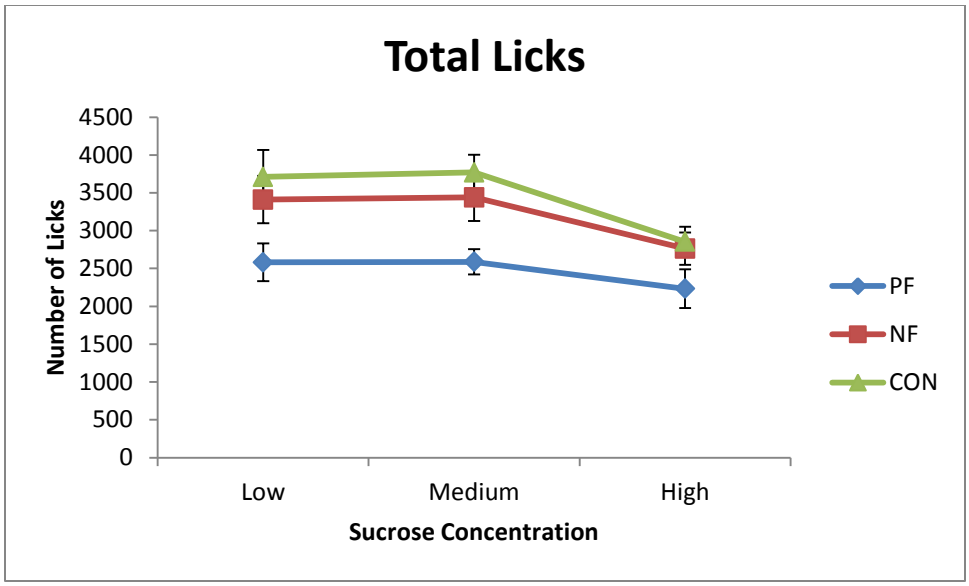

Figure 9: Total number of licks of sucrose per session, according to sucrose concentration

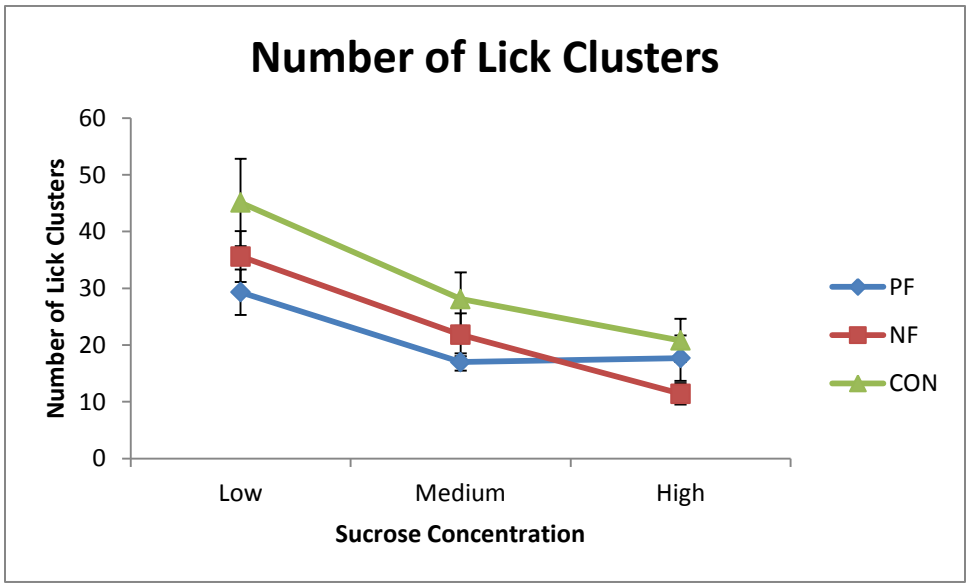

Figure 10: Total number of lick clusters per session, according to sucrose concentration

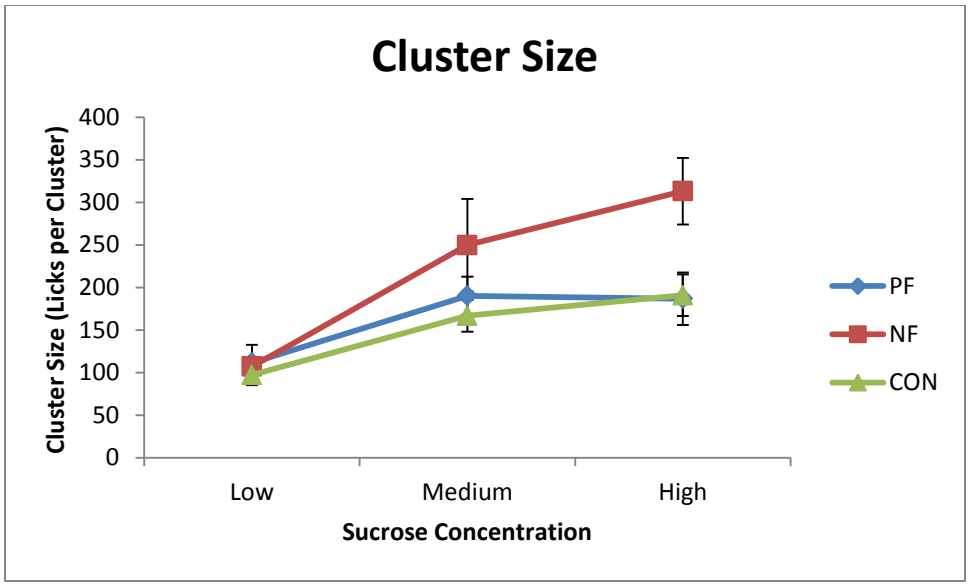

Figure 11: Size of lick clusters per session, according to sucrose concentration

Discussion

An overall effect of the processed foods cafeteria diet, natural foods cafeteria diet, and chow-only control diet was a significant difference in body weight. After three months on the experimental diets and before any testing began, PF rats were significantly heavier than rats in the other two groups. This finding was meant to illustrate that cafeteria diets, specifically those consisting of typical human foods, cause weight gain in rats. While there is a possibility that this weight gain affected the behavior of rats in the subsequent experiments, the effect of weight gain was not the main focus of this research. Rather, this study was designed to analyze the effects of variety and flavor-nutrient confusion incorporated into cafeteria diets, with implications for induced obesity from these factors. Results obtained from NF rats, who were similar in body weight to CON rats yet exhibited behavioral changes similar to PF rats, demonstrate that variety

is not a chief cause of becoming overweight. Furthermore, the implications of modified behaviors found in non-overweight rats (NF) indicate that behavioral effects caused by a cafeteria diet cannot be due entirely to differences in body weight.

The Flavor-Nutrient Conditioning tests that we conducted were designed to analyze whether a history of being on a natural or processed foods cafeteria diet influenced a rat's ability to learn new flavor-nutrient relationships. This was accomplished by giving rats repeated exposure to two equally preferred solutions: a high caloric density solution (6.5% glucose) and a low caloric density solution (1% glucose/ 0.125% saccharin), each of which were paired consistently with a flavor. Rats were then tested on their preference between the two flavors, as well as their acceptance of those flavors relative to unflavored solution. The first flavor-nutrient conditioning test measured preference between the CS+ and CS-; while all rats learned proficiently well to prefer the CS+, rats on the control diet showed a significantly higher preference for the CS+ than rats on the cafeteria diets. However, after gaining more experience with two new flavors and the calorie-paired and -unpaired solutions in FNC2, the higher level of proficiency in learning by CON rats was not upheld. Rather, FNC2 revealed that PF rats, followed by NF rats, learned a stronger relationship between the CS+, CS-, and US's as compared to CON rats. This was indicated by results from one-bottle acceptance in FNC2, in which PF rats drank more CS+ flavored solution than CS- or unflavored solution, NF rats drank more CS+ solution and more CS- solution

than unflavored solution, and CON rats did not have significantly different intakes between the three solutions.

The results from FNC2 indicate that PF rats learned flavor-nutrient relationships more effectively when acceptance is considered. Along with preference, acceptance is the other main effect of flavor-nutrient conditioning (Myers & Sclafani, 2006). Since FNC2 involved more extensive flavor-nutrient training than FNC1, perhaps PF rats need more experience with a flavor in order to maximize their flavor-nutrient conditioning. This would indicate that PF rats learn slower but better than CON rats, and NF rats fall somewhere in between. These findings were inconsistent with the null results obtained by Perez, Fanizza, and Sclafani (1999) that experience on a cafeteria diet did not change rats' ability to form flavor-nutrient associations. Our results also contradicted the results of a cafeteria diet study done by Naim, Brand, Kare, and Carpenter (1985), in which it was concluded that the high-fat high-sugar aspects of a diet had a much larger influence than the effect of variety on energy intake and weight gain. The results of the NF group, which were intermediate between those of PF and CON rats, indicate that variety does influence flavor-nutrient learning and thus has implications for energy intake, because the NF diet did not have high-fat high-sugar components.

One explanation for the more successful flavor discrimination by the PF group in FNC2's one-bottle acceptance test is that the PF diet necessitates that rats become more proficient at discriminating between flavor-nutrient relationships. This hypothesis is contrary to the flavor-confusion hypothesis that

inspired this research, which was that a processed food diet would impair new flavor-nutrient learning. Instead, maybe experience on a diet with so many confusing flavor-nutrient inconsistencies makes discriminating between similar stimuli a more beneficial behavior. In order to regulate their body weight, it is possible that PF rats with this confusing processed foods diet learn from their experience, rather than being impaired by it. In light of this new hypothesis, NF rats would have (and did) performed at a level intermediate between PF and CON rats, because they could have learned from their history of eating a variety of foods, yet they did not receive the experience with inconsistent flavor-nutrient relationships that was characteristic of the PF cafeteria diet. The idea that a flavor-nutrient inconsistent diet prepared PF rats for future flavor-nutrient conditioning is consistent with the easy-to-hard effect. The easy-to-hard effect describes how training on a simple task prepares an animal to do better on a subsequent more difficult task than if the animal was given the difficult task directly (Scahill & Mackintosh, 2004). In this study, the processed food cafeteria diet might have served as a preparatory flavor-nutrient discrimination task that prepared PF rats to learn more effectively in flavor-nutrient conditioning than rats not given the initial processed foods experience.

During Flavor-Nutrient Conditioning studies, we consistently noticed that cafeteria rats (PF and NF) drank smaller quantities of sweet solutions than CON rats. To confirm this finding, we examined intake of a sweet yet low-calorie solution of 2% sucrose/ 0.2% saccharin in the Ad Lib Sweet Consumption

paradigm. Results did confirm that PF rats and NF rats drank significantly lower quantities of the solution than CON rats.

The difference in sweet intakes across groups could have been explained by two factors: the groups might have differed in their level of motivation to obtain a sweet stimulus, or they might have perceived the palatability of sweet stimuli differently. To explain the results of the Ad Lib Sweet Consumption paradigm, we next conducted a Progressive Ratio test. For the PR test, rats were first shaped to consistently press a lever for a sucrose pellet reward. Lever pressing was then programmed to be on a progressive ratio schedule, such that the first pellet was earned by one lever-press and each subsequent pellet required an increasing number of lever-presses. The breakpoint of Progressive Ratio, which is typically defined as the number of lever-presses required for the final pellet earned by a rat in a PR session, was analyzed in terms of the number of pellets earned, the number of total lever presses, and the total session time per rat per session. All of these measures showed no significant differences across groups. The lack of differences between groups in PR testing found here contradicts the results of la Fleur *et al.* (2007), who found that consumption of a high-fat high-sugar diet caused rats to have higher breakpoints in progressive ratio testing, indicating a higher motivation for sucrose. Another study that found an effect of a high-fat diet on food motivation discovered that length of time on a diet can cause differences between groups to become significant, but the three months of dietary experience in the current study far surpassed the critical value of the high-fat study (Tracy *et al.,* 2015). A possible explanation for the lack of a difference in

motivation for sucrose in the current study is that while previous studies such as la Fleur *et al.* (2007) and Tracy *et al.* (2015) provided rats with unlimited access to their high-fat or HFHS diet, our rats had access to restricted amounts of cafeteria foods in their diets. More research is needed to examine the effects of dietary manipulations on food motivation.

One explanation for the lack of significant results in the Progressive Ratio test could be that rats raised under different dietary conditions (PF cafeteria diet, NF cafeteria diet, or chow-only control) are not differently motivated to work for a sucrose reward. This would mean that whatever behavioral changes a cafeteria diet induces, motivation for sucrose is not one of them. Another possible explanation for the similar results across groups in PR could be that progressive ratio testing was simply tested too long after the experimental manipulation (dietary conditions). Rats in this study were kept on their respective diets for 3 months, but progressive ratio testing took place after the rats had been off their diets for about 2 months. In the time period between the end of the experimental diets and the beginning of progressive ratio testing, the rats were exposed to several tests in which all three groups had near-identical experiences with different stimuli. It is possible that the effect of the experimental diets on a behavior, such as motivation to work for sucrose, wore off before the rats were tested on a PR schedule. Perhaps if PR testing had been conducted immediately after the end of the experimental diets, there would have been significant differences in different groups' breakpoints.

The possibility that the discovery of null results for Progressive Ratio testing was due to the diminution of the cafeteria diets' effects brings up an interesting point. How long might we expect behavioral changes caused by a natural foods or processed foods cafeteria diet to last? Are these changes worth researching if they are as fleeting as the results from PR testing might suggest? Some research has shown relatively long-lasting effects of cafeteria diets, such as the persistence of two weeks of a cafeteria diet affecting sensory-specific satiety one week later (Reichelt *et al.*, 2014). However, it is possible that different behavioral effects of a cafeteria diet can have varied durations. If a cafeteria diet does produce behavioral changes, but these changes weaken rapidly with time, then this finding has major implications for human recovery from processed food diets**.** Any potential dysregulation of body weight caused by a modern processed foods diet could be eliminated after a short period of time back on a natural diet if this hypothesis is correct.

Progressive Ratio testing was conducted to determine if a difference in motivation for sweet stimuli was causing the consistent difference in sweet intake across groups. Motivation was not significantly different between groups, so we next looked to the other possible cause of different intakes: palatability. To examine whether rats perceived the palatability of sweet solutions differently according to their diet history, the next test was lick microstructure. The lick microstructure test consisted of giving rats access to one bottle per session of a low (5%), medium (10%), or high (30%) concentration of sucrose solution. While the rats licked the solution, computer software recorded the timing and number of

licks so that each rat's licks could be analyzed in terms of clusters. Analysis of total licks in a session confirmed that control rats had the highest intake of all solutions, followed by NF rats and then PF rats. Analysis of cluster size, where larger clusters indicate higher perceived palatability, showed that NF rats found higher concentrations of sucrose to be significantly more palatable than did PF or CON rats. Finally, there was no difference between groups in the number of lick clusters, which is typically indicative of satiety.

The large difference in cluster size, which is interpreted as palatability, between NF rats and CON rats is an important finding because it raises more questions about why experience on a natural foods diet makes a high concentration of sucrose so palatable. Throughout their experience on the natural foods cafeteria diet, the only sugars that NF rats experienced were in foods such as fruits, which contained natural levels of sugars. Why might NF rats perceive sucrose to be more palatable than other rats? One potential reason is that NF rats are experiencing a contrast effect between sweet solutions, such as sucrose, and the foods received in their cafeteria diet. Over the course of their experience on the cafeteria diet, NF rats received a variety of fruits, grains, legumes, and vegetables. Vegetables, which were a large proportion of their supplementary diet, are often high in compounds that carry a bitter taste. It is possible that in comparison to the bitter tastes that NF rats remembered from vegetables during their cafeteria diet, sucrose solutions seemed extra palatable. This explanation is consistent with a positive contrast effect that could occur between bitterness in vegetables and sweetness of sugar solutions (Flaherty & Largen, 1975). The

existence of a contrast effect would explain the significantly larger cluster sizes in response to high concentrations of sucrose that was demonstrated by NF rats in comparison to PF and CON rats.

The combination of results between the Ad Lib Sweet Consumption paradigm and Lick Microstructure analysis produce a puzzling enigma. NF rats perceive high concentrations of sucrose to be even more palatable than do the PF and CON rats, whereas PF rats find sucrose to be just as palatable as the CON rats. However, these rats in the NF and PF groups are all drinking significantly less than CON rats when given access to sweet solutions, despite their equal or heightened perceived palatability of sweet solutions. The reasoning behind the reduced intake of sweet solutions in cafeteria diet raised rats is thus uncertain, but it raises new questions about the effect of cafeteria diets. The difference in perceived palatability is particularly important for NF rats compared to CON rats, because NF rats were simply raised on a large variety of straightforward, natural foods. There are clearly additional behavioral mechanisms being modified by the experience of variety in a cafeteria diet, and this is the first study to demonstrate this effect of variety separately from the effects of high-fat high-sugar components and flavor-nutrient confusion.

Overall, the processed and natural foods cafeteria diets implemented in this study caused two significant behavioral changes. First, flavor-nutrient conditioning showed that with enough experience, PF rats with a history of flavor confusion were certainly not impaired in learning new flavor-nutrient relationships. Rather, PF and NF rats learned just as well and perhaps better than

CON rats. Second, a history of being on the cafeteria diets caused rats to consume smaller amounts of sweet-tasting solutions compared to control rats. This effect was not found to be due to a different motivation to earn a sweet reward, and it also was not due to cafeteria diet rats perceiving sweet stimuli as being less palatable. Instead, NF rats found high concentrations of sucrose to be significantly more palatable than PF or CON rats. The finding that rats given experience on a cafeteria diet consistently consumed less sweet solutions in tests is currently an unexplained phenomenon that should be elucidated in future studies.

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Appendix

Food	Cage Serving (g)	kcal/g	Carbs/ Serving (g)	Protein/ Serving (g)	Fat/ Serving (g)
NATURAL FOODS					
Almonds	20	5.79	4.3	4.2	10
Apple	30	0.52	4.1	0.1	0.1
Arugula	15	0.25	0.5	0.4	0.1
Banana	35	0.89	8	0.4	0.1
Barley	25	3.03	18.2	2.3	0
Beets	35	0.33	2.6	0.3	0
Beets	35	0.33	2.3	0.3	0
Blueberries	30	0.51	3.7	0.1	0.2
Broccoli	20	0.26	1	0.6	0.1
Brown rice	30	3.54	22.5	2.5	0.9
Brussel sprouts	35	0.41	2.8	1.3	0.1
Buckwheat	40	0.92	8	1.4	0.2
Bulgar wheat	30	3.5	22.5	3.8	0.4
Butternut squash	35	0.45	4.1	0.4	0
Cabbage (red)	20	0.31	1.5	0.3	0
Cactus pear	35	0.41	3.3	0.2	0.2
Cantaloupe	35	0.34	2.9	0.3	0.1
Carrot	20	0.36	1.6	0.2	0.1
Cauliflower	40	0.24	1.9	0.8	0.1
Chicken	25	1.07	0.4	5.4	0.4
Coconut	12	6.67	3.2	0.8	8
Corn	30	0.88	6.2	0.9	0.2
Cucumber	40	0.15	1.5	0.3	0
Currants	15	3.25	11.6	0.4	0
Egg (scrambled)	30	1.49	0.5	0	3.3
Farro	30	3.49	21.3	3.6	0.5
Flax	15	3.64	11.5	1.9	0.8
Garbanzo beans	30	0.85	4.6	1.6	0.2
Grape nuts	20	3.45	16.6	2.1	0.3
Grapes	50	0.69	9.1	0.4	0.1
Green beans	25	0.39	1.9	0.4	0.1
Green pepper	20	0.2	0.9	0.2	0
Grits	35	3.66	27.3	3.4	0.4
Honeydew melon	35	0.36	3.2	0.2	0
Jasmine Rice	20	0.97	4.2	0.4	0
Kale	15	0.49	1.3	0.6	0.1
Kidney beans (red)	20	0.85	2.8	1.4	0
Kiwi	35	0.61	5.1	0.4	0.2
Lentils	25	3.52	15.8	6.2	0.3
Lettuce	20	0.14	0.6	0.2	0

Food	Cage Serving (g)	kcal/g	Carbs/ Serving (g)	Protein/ Serving (g)	Fat/ Serving (g)
NATURAL FOODS, cont.					
Lima beans	20	1.32	5	1.5	0.1
Liver	20	1.25	0.7	3.6	0.8
Mango	35	0.6	5.2	0.3	0.1
Millet	5	4	3.7	0.7	0.2
Mushrooms (portobello)	25	0.22	1	0.5	0.1
Mushrooms (white)	20	0.22	0.7	0.6	0.1
Nectarines	40	0.44	4.2	0.4	0.1
Oats	15	3.75	10.1	1.9	1.1
Okra	25	0.3	1.7	0.4	0.1
Papaya	40	0.43	4.3	0.2	0.1
Parsnips	30	0.75	5.4	0.4	0.1
Peaches	35	0.39	3.3	0.3	0.1
Peanuts	15	5.67	2.4	3.9	7.4
Pears	30	0.57	4.6	0.1	0
Peas	30	0.77	4.1	1.6	0.1
Pecans	15	7	2	1.5	11
Pine nuts	15	6.73	2	2.1	10.3
Pineapple	35	0.5	4.6	0.2	0
Pinto beans	30	0.82	4.6	1.4	0.2
Plums	40	0.46	4.6	0.3	0.1
Potatos	30	0.42	2.5	0.2	0
Prunes	25	2.5	16.3	0.6	0
Pumpkin seeds	20	5.59	2.1	6	9.8
Quinoa	25	3.6	16.9	3.5	1.5
Radicchio	20	0.23	0.9	0.3	0.1
Raisins	25	3	20	0.6	0
Raspberries	30	0.52	3.6	0.4	0.2
Rutabaga	35	0.37	3	0.4	0.1
Salmon	25	1.43	0	4.8	2
Sesame seeds	10	5.73	2.3	1.8	5
Shredded Wheat	15	3.47	12.2	1.8	0.3
Soy beans	20	1.22	1.9	2.6	1.3
Spinach	20	0.23	0.7	0.6	0.1
Squash (yellow)	35	0.19	1.4	0.4	0.1
Strawberries	30	0.35	2.7	0.1	0
Sugar Snap Peas	25	0.31	1.7	0.5	0.1
Sunflower seeds	20	5.84	4	4.2	10.3
Teff	25	3.6	18.5	3.5	0.5
Tomatillos	30	0.32	1.8	0.3	0.3
Tomatoes	30	0.18	1.2	0.3	0.1
Tuna	20	1.25	0	5.7	0.2
Walnuts	20	6.19	1.9	4.8	11.9
Watercress	15	0.11	0.2	0.3	0
Watermelon	35	0.3	2.6	0.2	0.1
Wax beans	25	0.17	0.8	0	0
Zucchini	35	0.17	1.1	0.4	0.1

Food	Cage Serving (g)	kcal/g	Carbs/ Serving (g)	Protein/ Serving (g)	Fat/ Serving (g)
PROCESSED FOODS					
Almond Granola	20	4.52	13.3	1.9	3.8
Apple Bars	30	3.5	22.5	0	1.5
Apple Granola	20	3.81	12.4	1.4	2.9
Apple Jacks	10	3.93	8.9	0.4	0.4
Apple Pie Filling	40	1.06	10.4	0	0
Baked Beans	20	1.45	6	1.2	0.2
Banana Muffins	20	3.83	11.1	0.9	3.4
Brown bread	25	2.29	12.8	1.3	0.2
Butterscotch chips	20	5.71	12.9	0	6.4
Candy Corn	20	3.59	17.9	0	0
Cap'n Crunch	10	4.07	8.5	0.4	0.6
Cheerios- Honey Nut	10	3.93	7.9	0.7	0.5
Cheerios- Peanut Butter	10	3.89	8.2	0.7	0.6
Cheese- cheddar	20	4.06	0.3	4.8	6.8
Cheese- Havarti	25	4.24	0	4.5	9.1
Cheese- Swiss	20	3.8	1.1	5.4	5.6
Cheesy Burger Macaroni	35	0.67	4	1.1	0.3
Cherry Pie Filling	40	1.18	11.8	0	0
Cinnamon Granola	20	4.36	15.3	1.5	2.5
Cinnamon Toast Crunch	10	4.04	7.9	0.5	1.1
Cocoa Pebbles	10	4	8.5	0.5	0.4
Cookies 'n' creme cereal	10	4.07	7.8	0.4	1.1
Corn Muffin	30	3.05	15.3	1.8	2.5
Corned Beef	20	2.32	0	5	2.9
Cracker Sandwiches- Cheddar Cheese	25	4.94	15.3	2.4	5.9
Cracker Sandwiches- Chocolate & Peanut Butter	15	4.72	9.8	1.2	2.8
Cracker Sandwiches- Cream Cheese & Chives	25	5.13	15.4	1.9	6.4
Cracker Sandwiches- Grilled Cheese	25	4.87	16	1.9	5.8
Cracker Sandwiches- Peanut Butter	15	5.21	8.6	1.6	3.7
Croutons	11	4.29	7.9	1.6	1.6
Croutons	11	4.29	7.9	1.6	1.6
Donuts (powdered)	20	4.15	10.9	0.8	4.2
Doritos Cool Ranch	10	5.36	6.4	0.7	2.9
Doritos Nacho Cheese	10	5	5.7	0.7	2.9
Fettucini Alfredo	30	1.11	6	1	0.6
Fig Bars	30	3.5	22.5	0	1.5
French fried onions	10	6.43	4.3	0	5
Frosted Flakes	10	3.67	8.8	0.5	0
Fruity Pebbles	10	4.04	8.6	0.5	0.4
Fudge Grahams	15	4.52	11.1	0.5	2.9
Funyons	8	5	5.1	0.6	2
Golden Puffs	15	4.07	13.3	1.1	0
Goldfish crackers-Cheddar	15	4.67	10	1.5	2.5
Goldfish crackers-Parmesan	25	4.67	16.7	2.5	4.2
Goldfish crackers-Pizza	15	4.52	9.7	1.5	2.4
Goldfish grahams-Honey Bun	15	4.67	11	1	3

Food	Cage Serving (g)	kcal/g	Carbs/ Serving (g)	Protein/ Serving (g)	Fat/ Serving (g)
PROCESSED FOODS, cont.					
Goldfish grahams-S'mores	15	4.67	11.5	1	2.3
Goldfish grahams-Strawberry Shortcake	15	4.67	11	1	2.5
Goldfish grahams-Vanilla Cupcake	15	4.67	10.5	0.5	2.5
Grahams	20	4.33	13.3	0.7	3.3
Honeybuns	25	4.6	13	1	6.5
Hummus Crisps-Caramelized Onion	10	4	7	1	1.2
Mac and Cheese	35	0.91	4.2	1.1	1.2
Maraschino Cherries	15	2	6	0	0
Marshmallow bits	10	3.88	9.6	0.2	0
Oreo cookies	25	4.71	18.4	0.7	5.1
Peanut butter crunch cereal	10	4.07	7.8	0.7	0.9
Peanut Butter Granola	20	4.36	12	3.6	2.5
Peanuts (Cocoa)	20	5.71	6.4	4.3	8.6
Peanuts (Salted caramel)	20	5.71	6.4	4.3	8.6
Pecan cakes	30	3.57	17.1	1.1	3.8
Pecan Granola	20	4.52	13.3	1.9	3.3
Pierogies (Four cheese)	35	1.87	10.8	1.9	1.9
Pizza (4 Cheese, frozen)	20	2.3	5.3	1.5	2
Pop Tarts- Blueberry	25	3.85	18.3	1	2.4
Pop Tarts- S'mores	25	3.85	17.3	1.4	2.4
Potato Chips	12	5.36	6	0.9	3.9
Potato Chips- Honey Mustard	10	5.36	5.4	0.4	3.2
Potato sticks- sour cream & onion	15	5.36	7.5	1.1	5.4
Pretzels- honey mustard onion	15	5	9.1	1.1	3.2
Pretzels	20	3.93	15.7	2.1	1.1
Pretzels- maple	20	4.79	20.7	4.3	0.4
Pringles-BBQ	15	5.36	8	0.5	4.8
Pumpkin Muffins	20	3.83	10.6	0.9	3.4
Pumpkin Pie Filling	40	1.03	9.2	0.5	0.2
Raspberry Granola	20	4.55	14.5	1.5	2.9
Raspberry Juicy Twists	25	3.42	20.4	0.7	0
Refried Beans	30	0.75	4	1.3	0.1
Root Beer Twists	25	3.16	19.1	0.7	0
Sausage links	30	2.89	0	5.3	6.7
Slim Jims	15	5	2.3	3.3	6.1
Snickerdoodle Cookies	20	4.44	14.1	0.7	3
Spaghettios	40	0.67	5.6	1	0.2
Strawberry Cream Wafers	12	4.69	8.3	0.4	2.6
Strawberry Tasty Twirls	25	3.42	20.4	10.5	0
Sweet Potato Casserole	35	1.35	11.3	0.5	0
Sweet Potato Chips	10	4.64	7.9	0.4	1.8
Sweet Potato Tater Tots	30	1.67	8.2	0.4	1.6
Tortilla Chips	15	5.36	10.7	1.6	3.8
Velveeta	25	2.86	2.7	3.6	4.5
Vienna sausage	25	2.33	1.7	2.1	5
Wheat Thins	25	4.52	17.7	1.6	4